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A comparison of gas chromatography and differential respirometer methods to measure soil respiration and to estimate the soil microbial biomass

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1. Introduction

Gas chromatography (GC) is a sensitive, rapid, and non-destructive method to detect CO_2 in the atmosphere (PUTNAM, 1976). The method has been used to measure the CO_2 respired from soil (SPARLING, 1981) to obtain the basal rate of microbial respiration, and also to estimate the soil microbial biomass by the substrate-induced-respiration (SIR) method (SPARLING, 1981) and the fumigation-incubation method (LYNCH & PANTING, 1980).

The measurement of respiration by the GC method usually involves incubating soil in a sealed container with a gas sampling septum. The headspace gases are sampled after various intervals and the rate of CO_2 accumulation gives the rate of soil respiration. This method has been criticised as being at risk of underestimating soil respiration because it assumes equilibrium between the gaseous and solution phases within the system. When used with soils of high *p*H or when the volume of solution is large in relation to the headspace volume, a high proportion of the CO_2 may be retained in the solution phase, and sampling of headspace CO_2 alone will underestimate the rate of respiration (MARTENS, 1987).

The amount of CO_2 dissolved in solution depends primarily on the aqueous carbonate equilibrium: CO_2 (gas) + $H_2O \rightleftharpoons [H_2CO_3] \rightleftharpoons [H^+] + [HCO_3^-] \rightleftharpoons [2H^+] + [CO_3^{2+}]$. At the *p*H of most biotical reactions (below 8), the amount of $[CO_3^{2-}]$ formed is negligible; the main controlling equilibrium is the amount of $[HCO_3^-]$ that is formed. This amount, which is *p*H dependent, is described by the Henderson-Hasselbach equation:

$$pH = pK + \frac{\log [HCO_3^-]}{[CO_2]}$$

The relationship shows that the amount of CO_2 retained in the solution phase increases as the *p*H rises, with a sharp increase in retention above *p*H 6.5. The potential for large amounts of CO_2 to remain in solution was the reason that WEST & SPARLING (1986) recommended that their modified SIR method (which uses a soil-solution slurry) was not suitable for use on soils of $pH > 6.5$. Under conditions where the volume of soil solution is small and the $pH < 6.5$, then the amount of CO_2 remaining in solution is much smaller and the underestimation is correspondingly less.

These conclusions were based upon the theoretical equilibrium of CO_2 between the gas and solution phases. However, equilibria can be very slow to establish in soil even under a system where the CO_2 is flushed out of soil by a stream of air (MARTENS, 1987). Further, simple equilibria between CO_2, *p*H and HCO_3^- are complicated in soil by the formation of Ca, Fe and Mg bicarbonates. Consequently, the theoretically-derived equilibria may not apply under practical experimental conditions.

We report here on a comparison of soil respiration (CO_2 production) measured by GC, with soil respiration (CO_2 production and O_2 uptake) measured by Gilson Differential Respirometer. The

respirometer method incorprates alkali traps to absorb CO_2 from the gaseous phase and the flask design provides a large solution-gas interface. This strong sink for CO_2 ensures that retention of CO_2 in the soil solution is minimal (UMBREIT *et al.*, 1964). The distribution of CO_2 under the two systems was measured and compared with the theoretical equilibria. The reliability and limitations of the two methods are discussed.

2. Materials and methods

2.1. Respiration measurements

Respiration was measured by the "static" GC method and by Differential Respirometer. One set of respiration measurements by the GC method was made under the conditions of the modified SIR assay (WEST & SPARLING, 1986). Other combinations of soil-solution-container volume were chosen as in table 1, to check for effects of headspace and solution volumes on CO_2 retention. The McCartney bottles (4 replicates per assay) containing the soil and solution were sealed with Vacutainer stoppers (Becton-Dickinson, Boston. Ma) and incubated at 25 °C. Samples of headspace gases (1 ml) were taken via the stopper with a hypodermic needle and 2 ml syringe. The gas was sampled 30 min and 150 min after adding the glucose solutions, with vortex mixing for 5 s immediately before taking the gas samples (WEST & SPARLING, 1986). The CO_2 concentration was measured using a Carle 8500 gas chromatograph with a Poropak T column, He carrier gas and thermal conductivity detector. The concentration of CO_2 in the headspace (% v/v) was converted to $\mu g\, g^{-1}\, h^{-1}$ assuming that no CO_2 was present in solution – i.e. effective headspace volumes of 26 or 18 ml respectively, for the McCartney bottles containing either 2 or 10 ml of solution plus 1 g soil (equivalent volume about 0.5 ml) (table 1). The concentration of glucose was 30 mg ml^{-1} (WEST & SPARLING 1986).

Table 1. Container type and volume, mass of soil, and solution and headspace volumes of treatments used to assess soil respiration.

Treatment	Container type	Total volume [ml]	Mass of soil [g]	Solution volume [ml]	Headspace volume [ml]	Comments
1	McCartney bottle	28.5	1.0	2	26	5 s shake
2	McCartney bottle	28.5	1.0	10	18	before sampling
3	Gilson flask	19.0	0.5	1	17.8	Shaken
4	Gilson flask	19.0	0.5	5	13.8	continuously

Table 2. Equilibrium reactions between CO_2 and H_2O at 25 °C (LINDSAY, 1978).

Reaction	Equilibrium	log K°
1	CO_2 (gas) + $H_2O \rightleftharpoons H_2CO_3^\circ$	− 1.46
2	$H_2CO_3^\circ \rightleftharpoons H^+ + HCO_3^-$	− 6.36
3	$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$	−10.33
4	CO_2 (gas) + $H_2O \rightleftharpoons H^+ + HCO_3^-$	− 7.82
5	CO_2 (gas) + $H_2O \rightleftharpoons 2H^+ + CO_3^{2-}$	−18.15

O_2-uptake and CO_2-production were also measured using a Gilson (constant pressure) Differential Respirometer. The instrument had 14 flasks of 19 ml nominal volume. They were shaken throughout the assay at 100 strokes min^{-1}, and the water bath temperature was 25 °C. Duplicate flasks were run of each treatment (table 1), the blank flasks contained no soil. To measure O_2 uptake, the centre well of the flask contained 0.3 ml of *M* KOH to absorb the CO_2 with a small fan of glass-fibre filter paper to increase the area of absorptive surface. To measure CO_2 production, the "direct" method (UMBREIT *et al.*, 1964) was used with one set of flasks (in duplicate) measured in parallel with those used for O_2 uptake, with KOH omitted from the centre well. All flasks were equilibrated with

shaking for 30 min after adding the glucose solution, and the volume changes measured every 30 min subsequently by the directly-calibrated micromanometers.

2.2. Soil

A range of samples with varying *p*H was produced by adding $Ca(OH)_2$ to an acid pasture soil of low fertility. The soil, Pomare, is a silt loam with a *p*H (in water) of 4.21 and with 4.6% C and 0.36% N. Topsoil samples (0–75 mm) were taken with an auger (25 mm diameter) and 50 cores bulked together. The surface vegetation was removed and the soil sieved <2 mm while field moist (28% w/w). The $Ca(OH)_2$ was well-mixed with the subsamples of soil, the moisture content adjusted to 40% m/m (−5 kPa potential), and incubated at 25 °C for 15 d to allow equilibration.

The *p*H of the soil slurry was measured *in situ* after the soils had been mixed with the glucose solution and after the final (150 min) gas sample had been taken. It was not possible to measure the solutions in the Gilson flasks because the design left insufficient space to insert the glass *p*H electrode. Consequently because the soil-to-solution ratios were the same, it has been assumed the *p*Hs of the soil-glucose solutions in the Gilson flasks were the same as those in the McCartney bottles.

2.3. Theoretical equilibria

The equilibrium reactions between CO_2, water and the various carbonate species are shown in table 2 (LINDSAY, 1979). For this study the amount of CO_2 dissolving in water (reaction 1) and the amount of bicarbonate (HCO_3^-) formed (reaction 4) are the main points of interest. Under the *p*H conditions of most biological systems the amounts of carbonate (CO_3^{2-}) formed (reactions 3 and 5) are negligible and can be ignored.

The worked example below shows the calculation of the theoretical distribution of CO_2 between the gaseous and solution phases in a container with 26 ml headspace, 2 ml of solution at *p*H 6.0, a CO_2 concentration in the headspace of 0.1% (v/v) at a temperature of 25 °C.

(i) CO_2 in the gas phase

$$N_{CO_2} = \frac{pCO_2 V_g}{RT}$$

Where V_g = headspace volume (cm^3), T = temperature (°K), R = the gas constant (82.0575 cm^3 atmospheres $°C^{-1}$ $mole^{-1}$) and pCO_2 is the partial pressure of CO_2 (% v/v).
Thus

$$N_{CO_2} = \frac{0.1 \times 26}{100 \times 82.0575 \times 298}$$

$$N_{CO_2} = 1.0632 \times 10^{-6} \text{ moles} \qquad [1]$$

(ii) CO_2 in solution phase.

The amount of CO_2 (moles) in solution depends on the equilibrium between $[H_2CO_3°]$ and $[HCO_3^-]$, (moles per litre)

$$N_{CO_2} = \frac{V_s}{1000}[H_2CO_3°] + \frac{V_s}{1000}[HCO_3^-]$$

where V_s = volume (cm^3).

For practical purposes $[H_2CO_3°]$ has been assumed to be equivalent to the activity of $H_2CO_3^-$ for the dilute soil solutions used in this study. A similar assumption has also been made for $[HCO_3°]$ and the activity of HCO_3^-, but it should be noted that where the ionic strength exceeds 0.01 *M*, then the activity of the species deviates significantly from the concentration (LINDSAY, 1979). The relationship for a dilute solution is thus:

$$\log [H_2CO_3°] = \log K° + \log pCO_2 \text{ (Reaction 1, table 2)}$$

(where pCO_2 is the partial pressure of CO_2)

rewrite as: $$H_2CO_3° = \text{antilog}\left[-1.46 + \log\left(\frac{0.1}{100}\right)\right]$$

$$H_2CO_3° = \text{antilog}\,[-4.46]$$

$$H_2CO_3° = 3.467 \times 10^{-5} \text{ moles} \qquad [2]$$

The amount of $[HCO_3^-]$ formed in the equilibrium depends on the *p*H value as shown by the Henderson-Hasselbach equation.

$$\log [HCO_3^-] = \log K^\circ + pH + \log pCO_2 \text{ (Reaction 4, table 2)}$$

rewrite as: $$[HCO_3^-] = \text{antilog} \left[-7.82 + pH + \log \left(\frac{0.1}{100} \right) \right] \quad \text{(from table 2).}$$

Thus at *p*H6: $[HCO_3^-] = \text{antilog } [-7.82 + 6 + -3.0]$

$$HCO_3^- = 1.514 \times 10^{-5} \text{ moles} \qquad [3]$$

Substituting the equilibria for $H_2CO_3^\circ$ and HCO_3^- (equations 2 and 3) into the original equilibrium equations we obtain:

$$N_{CO_2} = \frac{V_s}{1000} [(3.467 \times 10^{-5}) + (1.514 \times 10^{-5})]$$

For V_s = 2 ml of solution volume

$$N_{CO_2} = 9.961 \times 10^{-8}. \qquad [4]$$

Thus in the system described there would be 1.0632×10^{-6} moles in the gaseous phase and 9.961×10^{-8} moles in the solution phase, i.e. a distribution in favour of the gaseous phase by 10.7 : 1. The measurement of the headspace CO_2 alone would result in an under estimation of the total CO_2 in the system by 8.6%, or require a factor of 1.094 to correct for retention of CO_2 in the solution phase.

The distribution of CO_2 between the gaseous and solution phases was calculated for the conditions given in table 1, over the *p*H range 4−8. The retention factors necessary to convert from the gaseous CO_2 concentration to that present in the whole system are plotted graphically in fig. 1.

3. Results

3.1. Theoretical distribution of CO_2

Equilibrium calculations show there was the potential for considerable retention of CO_2 in the solution phase (table 3). Retention is less at low solution volumes (1−2 ml) and at *p*H<6 about

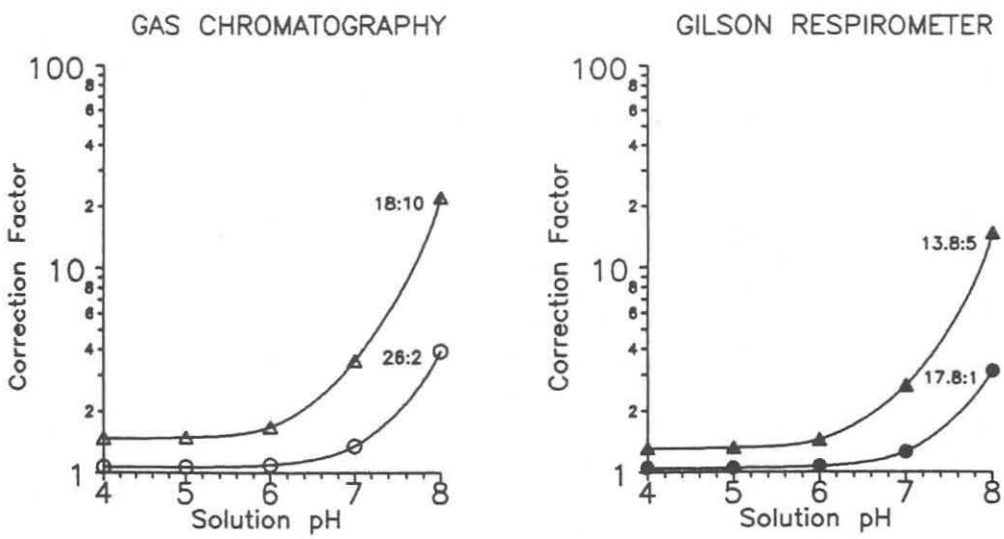

Fig. 1. Factors required to correct for CO_2 retention in the solution phase under two systems to estimate soil respiration. Ratios shown are the headspace: solution volume for the systems tested; curves are based on the theoretical equilibria predicted by the Henderson-Hasselbach Equation.

Table 3. Theoretical distribution of CO_2 between the gaseous and solution phases at various *p*H values, assuming a gaseous phase CO_2 concentration of 0.1% v/v.

Headspace [ml]	Solution [ml]	CO_2 in gaseous phase (moles)	CO_2 present in the solution phase (moles)[1])				
			*p*H4	*p*H5	*p*H6	*p*H7	*p*H8
26	2	1.063×10^{-6}	6.96×10^{-8} (1.07)+	7.24×10^{-8} (1.07)	9.96×10^{-8} (1.09)	3.72×10^{-7} (1.35)	3.09×10^{-6} (3.91)
18	10	7.36×10^{-7}	3.48×10^{-7} (1.47)	3.62×10^{-7} (1.49)	4.98×10^{-7} (1.68)	1.86×10^{-6} (3.53)	1.55×10^{-5} (22.03)
17.8	1	7.26×10^{-7}	3.48×10^{-8} (1.05)	3.62×10^{-8} (1.05)	4.98×10^{-8} (1.07)	1.86×10^{-7} (1.26)	1.55×10^{-6} (3.13)
13.8	5	5.62×10^{-7}	1.74×10^{-7} (1.31)	1.81×10^{-7} (1.32)	2.49×10^{-7} (1.44)	9.30×10^{-7} (2.65)	7.74×10^{-6} (14.77)

[1]) Figures in parenthesis show the "retention factor". This factor is used to estimate the total CO_2 in the system from that present in the gaseous phase alone (see text).

Table 4. Rates of O_2 uptake and CO_2 production, of soil samples at various *p*H and soil : solution ratios measured by a gas chromatography method or by Gilson respirometer.

Sample	*p*H	Soil : solution ratio	Rate of respiration ($\mu l\ g^{-1}\ h^{-1}$)		
			Gas chromatography	Gilson respirometer	
			CO_2	O_2[1])	CO_2[2])
1	4.98	1:2	10.33	24.4	20.1
2	5.30	1:10	7.46	25.1	17.1
3	5.08	1:2	13.95	24.4	24.2
4	5.47	1:10	8.99	23.5	20.9
5.	5.62	1:2	12.64	22.0	21.1
6	5.90	1:10	8.05	22.4	20.4
7	6.06	1:2	7.58	25.4	20.4
8	5.86	1:10	2.39	23.8	16.7
9	6.66	1:2	6.31	47.6	37.9
10	6.18	1:10	1.14	46.6	19.6
11	8.00	1:2	4.23	53.3	26.3
12	8.44	1:10	0.50	45.9	2.7
LSD	0.06	–	1.23	ND[3])	ND

[1]) Calculated using the direct method with KOH trap, figures are means of duplicate flasks.
[2]) Calculated by the direct method, not corrected for CO_2 retention.
[3]) ND = Not determined.

10% of the CO_2 may be in solution. However, with larger solutions volumes (5 ml and 10 ml) over 30% of the CO_2 could be retained in solution even at *p*H < 6. Above *p*H 6 the amounts present in solution can increase rapidly, so that at *p*H 8 between 3.1 and 22 times more CO_2 may be present in the solution phase compared to the gaseous phase, depending on the volumes of solution and headspace in the systems (table 3). Further calculation (not presented here) showed that with solutions of low concentration (below 0.1 *M*) the effect of ionic strength on the retention factors is small, particularly below *p*H 7, and error from this source can be disregarded.

3.2. Soil respiration

The rate of soil respiration estimated by the Gilson differential respirometer was consistently greater than that estimated by the GC method (table 4). Estimated by respirometer the O_2-uptake of the soils increased as the soil *p*H increased, with little apparent effect of the solution volume. In contrast, the CO_2 production appeared to decline as the soil *p*H increased, and was much decreased in the presence of larger volumes of solution (table 4). Below *p*H 6 and at soil : solution ratio of 1 : 2 the CO_2 production estimated by the GC method was roughly half of that estimated by respirometer.

Above *p*H 6 the divergence between the GC and respirometer measurements was even greater, particularly at soil : solution ratios of 1 : 10. The apparent decrease in CO_2 production, in the higher *p*H soils, and with the larger solution volumes suggested considerable amounts of CO_2 were being retained in the solution phase.

The CO_2 production for the GC and respirometer system was recalculated using the appropriate factors to correct for CO_2 retention (fig. 1). For the Gilson respirometer the corrected CO_2 production was very similar to O_2 uptake except for the two samples of high *p*H (samples 11 and 12), where the correction factors were very large with the potential for considerable error in the final recalculated figures (table 5). Omitting these high *p*H samples the respiratory quotients ranged from 0.7 to 1.3 (mean 0.98).

Table 5. Factors to correct for CO_2 retention in solution, the **corrected** CO_2 production ($\mu l\ g^{-1}\ h^{-1}$), and the respiratory quotients of soils at various *p*Hs, estimated using gas chromatography or Gilson respirometer.

Sample	Gas chromatography			Gilson Respirometer		
	Retention factor[1])	**Corrected** CO_2 production	Respiratory quotient[2])	Retention factor[1])	**Corrected** CO_2 production	Respiratory quotient[2])
1	1.07	11.1	0.45	1.05	21.1	0.86
2	1.50	11.2	0.45	1.32	22.6	0.90
3	1.07	14.9	0.61	1.05	25.4	1.04
4	1.53	13.8	0.59	1.36	28.4	1.21
5	1.08	13.7	0.62	1.07	22.6	1.03
6	1.64	13.2	0.59	1.45	29.6	1.32
7	1.09	8.3	0.33	1.07	21.8	0.86
8	1.62	3.9	0.16	1.40	23.3	0.98
9	1.17	7.4	0.16	1.13	42.8	0.90
10	1.75	2.0	0.04	1.56	30.6	0.66
11	3.90	16.5	0.31	3.13	82.3	1.54
12	48.0	24.0	0.52	34.0	91.8	2.00

[1]) From fig. 1, using the soil solutions *p*H to obtain the retention factor

[2]) Ratio of CO_2 produced/O_2 uptake. O_2 uptake was measured by Gilson respirometer

The recalculated data for CO_2 production measured by the GC method were consistently much less than those obtained by respirometer, and markedly so at the higher *p*H values (table 5). Assuming that the O_2 uptake was similar in both the GC and respirometer systems, then respiratory quotients (RQ) can be calculated for the GC method. These RQ values were very much lower than those obtained by the respirometer method, and ranged from 0.04 to 0.62 with a mean of 0.4. If the O_2 uptake measured by respirometer is accepted as a reliable measure of soil respiration, then the GC method underestimated CO_2 production, even after correction for the theoretical amounts of CO_2 retained in the solution phase.

4. Discussion

Equilibria calculations showed there was the potential for a considerable amount of respired CO_2 to remain in the soil solution under some of the experimental conditions tested. Measurement of the CO_2 production by both the GC method and by Gilson differential respirometer showed that at pH >6.5 and at high solution volumes, the amount of CO_2 respired by the soils apparently declined. This decline was not matched by a decrease in O_2 uptake, suggesting that the CO_2 was not being detected because it was being retained in the soil solution as predicted by the equilibria calculation. Under the conditions of the Gilson respirometer flasks, the distribution of CO_2 between the gaseous and solution phases was close to the theoretical equilibrium, and the CO_2 production, when corrected for CO_2 retention in solution, was close to the expected amount. However, under the conditions used to measure CO_2 by GC there was a consistent underestimation of the CO_2 production, as assessed using O_2 uptake and an RQ = 1 as a base line. The underestimation suggests that under the conditions of the GC assay the equilibria were biased in favour of the solution phase.

WEST & SPARLING (1986) recommended that vortex mixing of the soil slurry was necessary prior to sampling of the head space gases for the SIR biomass assay, but found that longer vortex mixing, or even continuous shaking did not cause any further significant increase in the concentration of CO_2 in the gaseous phase. These findings are not necessarily in conflict with the present results which indicated a high degree of CO_2 retention in the solution phase. It may be that the equilibria are slow to establish in the McCartney bottle system whatever the method to enhance the gas-solution interface. In contrast, the comparatively large gas-solution interface of the Gilson flask, with continuous shaking, established equilibria very similar to those predicted from the theoretical distribution of CO_2.

The retention of CO_2 in the soil solution when using the McCartney bottle system is of particular concern where the solution volume is high and of pH >6.5. In further studies WEST et al. (1989) compared the CO_2 production from field-moist soils (water content <0.6 ml g^{-1}) at their natural pH (pH 5.0–6.3) with the O_2 uptake measured by respirometer. Under these conditions the O_2 uptake and CO_2 production were very similar, giving respiratory quotients of around 0.9–1.0, suggesting that the retention of CO_2 in the soil solution was small. The majority of topsoils in New Zealand are acidic; of 561 samples tested by our analytical laboratory, 543 (97%) had a pH of ≤ 6.5 and 87% had a pH of <6.0. Consequently, under the usual acidity and moisture conditions pertaining in New Zealand, the GC method to measure CO_2 production will not be seriously in error when applied to these soils.

A more serious doubt is the reliability of the GC method when used to measure the CO_2 produced under the conditions of the modified SIR assay. WEST & SPARLING (1986) recommended adding glucose solution to the SIR assay in order to maintain adequate moisture and to control the substrate distribution and concentration. Under these conditions the static GC method greatly underestimates the total CO_2 production. However, this limitation is not so serious as may at first appear. The SIR method requires calibration of the respiration rate against an alternative measure of the microbial biomass. Consequently, if the method used to measure CO_2 during the SIR assay is consistent, then any under- (or over-) estimation of the respiration rate should be compensated by the calibration procedure. The modified SIR and GC method may not give a good measure of the absolute respiration, but if **consistent** should give a reliable measure of the microbial biomass C. This view is supported by the very good linear agreement between the modified SIR method and the fumigation-extraction and fumigation-incubation methods to estimate microbial C on a wide range of New Zealand soils (SPARLING & WEST, 1988a, b).

The consistency of the GC method applied to the SIR assay appears reasonable up to pH 6.5, although our results suggest an under-estimation of CO_2 production by about 50%. Our calibration between the respiration rate and microbial C, which takes into account this under-estimation, was: Microbial C (μ g g^{-1}) = 41 (μl CO_2 g^{-1} h^{-1}) + 13 (WEST *et al.*, 1986). If the microbial C was estimated using the O_2 uptake or the CO_2 production (corrected for any retention) measured by respirometer (i.e. no underestimation) our results suggest the calibration should be: Microbial C (μg g^{-1}) = 23 (μl O_2 g^{-1} h^{-1}). These calibrations are very similar to the relationships published by ANDERSON & DOMSCH (1978) for CO_2 viz: Microbial C (μg g^{-1} soil) = 40.04 (μl CO_2 g^{-1} h^{-1}) +

3.7 and by BECK (1984) for O_2: Microbial C ($\mu g\ g^{-1}$ soil) = 19.6 ($\mu l\ O_2\ g^{-1}\ h^{-1}$) (Note the units have been changed from their published form to make them consistent with the present paper).

In contrast to the CO_2 under-estimation by the GC method, MARTENS (1987) found that the intermittent flushing method using the Wösthoff Ultragas analyser was overestimating the CO_2 production. When applied to the SIR test he suggested a continual flushing method was needed to accurately measure respiration and a revised calibration was proposed: Microbial C ($\mu\ g^{-1}$) = 49.5 ($\mu l\ CO_2\ g^{-1}\ h^{-1}$) − 1.67. This increase seems surprising because, if the system was aerobic, then the respiratory quotient should be about 1.0 (UMBREIT *et al.*, 1964), and the amount of CO_2 respired should be similar to the O_2 uptake (i.e. about 20 $\mu l\ h^{-1}\ \mu g^{-1}$ microbial C). The high CO_2 output per unit of microbial C obtained by MARTENS (1987) is perhaps an indication that soil respiration had been stimulated by the flushing technique (SAKAMOTO & YOSHIDA, 1988; HIGASHIDA & NISHIMUNE, 1988). To obtain a reliable estimate of the microbial C by the SIR method, it is very important that the technique used to measure soil respiration should be calibrated against an alternative measure of the microbial biomass C.

5. Conclusions

The GC method to measure soil respiration can result in underestimation of the CO_2 production because CO_2 is retained in the soil solution. The retention is small where soils have a *p*H < 6.5, and where the volume of solution is small in relation to the headspace volume (ratio 1 : 20). Retention increases markedly above *p*H 6.5, and where large volumes of solution are present, such as during the modified SIR assay.

The GC method is satisfactory to estimate the respiration of soils with *p*H < 6.5 at field moisture capacity or less, and for use with the modified SIR method, provided an appropriate calibration has been established to convert from the rate of respiration to the microbial biomass C.

6. Zusammenfassung

[Ein Vergleich von Gas-Chromatographie (GC) sowie unterschiedlichen Methoden zur Messung der Bodenatmung und Schätzung der mikrobiellen Biomasse im Boden]

Die Löslichkeit von CO_2 in wäßriger Lösung birgt die Möglichkeit, Irrtümer bei der Messung der Bodenatmung (CO_2-Freisetzung) zu verursachen, besonders bei solchen Methoden, bei denen CO_2 sich im überstehenden Luftraum von verschlossenen Behältern ansammeln kann. Aus Kalkulationen, die auf der Henderson-Hasselbach-Gleichung basieren, ergibt sich die Voraussage, daß die CO_2-Retention in Lösungen mit einem *p*H oberhalb 6,5 zunimmt. Die Respirations-Raten von Böden mit einem *p*H von 4,9 bis 8,4 wurden gaschromatographisch gemessen (CO_2-Akkumulation wurde zugelassen) und mit der O_2-Aufnahme sowie mit der mit unterschiedlichen Respirometern (auf der Basis von Alkali-Absorption) gemessenen CO_2-Freisetzung verglichen. Unter Bezug auf theoretische Gleichgewichte für CO_2 berechnete Faktoren wurden für zwei Systeme abgeleitet und dazu verwendet, die Respirations-Raten um jene Beträge zu korrigieren, die sich aus der Zurückhaltung von jeglichem CO_2 in den Bodenlösungen ergeben.

Die mit Hilfe unterschiedlicher Respirometer gemessene (und für die CO_2-Retention korrigierte) Bodenatmung stimmte mit der O_2-Aufnahme gut überein (bei gegebenen Respirations-Quotienten um 1,0), wie das für ein Glukose-Substrat zu erwarten war. Im Gegensatz dazu war die gaschromatographisch gemessene CO_2-Freisetzung beständig geringer als die respirometrisch gemessene und gerade nach Korrektur für die CO_2-Retention war sie vom Volumen und dem *p*H der Bodenlösung ausgesprochen beeinflußt. Es scheint so, daß (unter den in dem für die Inkubation der Bodenproben verwendeten Gefäß-System gegebenen Bedingungen) gaschromatographische Messungen niedrigere Gesamt-Raten der Respiration ergaben und/oder daß Gleichgewichtszustände zwischen der Bodenlösung und dem (im geschlossenen Gefäß) überstehenden Luftraum sich eingestellt haben.

Es wird (daher) empfohlen, die gaschromatographische Methode nur zur Messung der Respiration von Böden zu verwenden, deren Saugspannung im Bereich (oder unterhalb) der Feldkapazität liegt und deren *p*H niedriger als 6,5 ist. Die gaschromatographische Methode zur Schätzung der mikrobiellen Biomasse (mikrobielles C_{org}) von der Rate der substratinduzierten Respiration (SIR) sollten ebenfalls auf die Messung von Böden unterhalb von *p*H 6,5 beschränkt werden. Die Irrtumswahrscheinlichkeit von Schätzungen des mikrobiell gebundenen Kohlenstoffs mit Hilfe der modifizierten SIR-Methode ist nicht groß, weil die zur Umrechnung der Respirations-Raten in mikrobiell gebundenen Kohlenstoff verwendete Eichung die geringere Effektivität der CO_2-Messung durch die GC-Methode berücksichtigte.

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(continued overleaf)

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SPARLING, G. P., & A. W. WEST, 1990. A comparison of gas chromatography and differential respirometer methods to measure soil respiration and to estimate the soil microbial biomass. Pedobiologia **34**, 103–112.

The solubility of CO_2 in aqueous solution has the potential to cause errors in the measurement of soil respiration (CO_2 output), particularly in those methods where CO_2 is allowed to accumulate in the headspace of a sealed container. Calculations based on the Henderson-Hasselbach equation predict that CO_2 retention increases markedly in solutions above *p*H 6.5. The rates of respiration of soils of *p*H 4.9 to 8.4 were measured using a gas chromatography method (CO_2 allowed to accumulate) and compared with O_2 uptake and CO_2 output measured by differential respirometer (CO_2 trapped in alkali). Factors calculated from the theoretical equilibria for CO_2 were derived for the two systems and used to correct the respiration to allow for any CO_2 retained in the soil solution.

The CO_2 respiration measured by differential respirometer and corrected for retention agreed well with the O_2 uptake, giving respiratory quotients around 1.0, as expected for the glucose substrate. In contrast, the CO_2 output measured by gas chromatography was consistently less than that estimated by respirometer, and even after correction for CO_2 retention, was markedly affected by the volume and *p*H of the solution. It appears that in the flask system used to incubate the soils for CO_2 measurement by gas chromatography, the overall rate of respiration was lower and/or equilibrium had not been established between the soil solution and headspace gases.

It is recommended that the gas chromatorgraphy method to measure soil respiration be restricted to soils at field capacity or below, and of *p*H <6.5. The use of the gas chromatography method to estimate the microbial biomass C from the rate of substrate-induced-respiration (SIR) should also be restricted to soils below *p*H 6.5. The estimation of microbial biomass C by the modified SIR method is not greatly in error because the calibration used to convert from the rate of respiration to the microbial biomass C takes into account the lower efficiency of measurement of the CO_2 by the GC method.

Keywords: carbon dioxide, soil respiration, gas solubility, *p*H, gas chromatography, differential respirometer, carbonate equilibria.

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